

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE 16 Apr 2002	3. REPORT TYPE AND DATES COVERED Final (01 May 1998 - 13 Aug 2000)		
4. TITLE AND SUBTITLE Nanoscale Field Localization for Manipulation and Probing of Computationally Interesting Biomolecules		5. FUNDING NUMBERS N00014-98-1-G-0677		
6. AUTHORS D. W. van der Weide				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Delaware (c/o University of Wisconsin) Department of Electrical and Computer Engineering 1415 Engineering Drive Madison WI 53706		8. PERFORMING ORGANIZATION REPORT NUMBER DVDW ONR		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research (Dr. Ira Skurnick)  800 North Quincy Street Arlington VA 22217-5000		10. SPONSORING / MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Unrestricted		12b. DISTRIBUTION CODE		
<b>DISTRIBUTION STATEMENT A</b> Approved for Public Release Distribution Unlimited				
13. ABSTRACT (Maximum 200 words) Several new and important results were enabled by this grant, which was to build a bioelectronic interface to single protein molecules. This interface consists of both environmental and mechanical supports for a membrane protein. While supported membranes are being studied in many laboratories, single channel probing has been performed almost exclusively with voltage-clamp techniques, by using fragile glass pipettes, or with cumbersome planar bilayer equipment unsuitable for field deployment. Single channel recording cannot yet be done on supported membranes. In this work, multifunctional scanned probe microscope tips were designed and fabricated to use in accessing the state of a membrane porin protein using microwave reflection and transmission, as well as fluorescent probing using photodiode probes. Specific accomplishments were: development of a micromachined probe process and several probes to perform combined scanning force microscopy together with microwave probing, development of a companion process for combined optical and scanning force probing, imaging of calcium waves in brain slices, and preliminary results on single-protein probing with both direct-current and high-frequency probes.				
14. SUBJECT TERMS Single-channel probing; Scanning probe microscope; near-field probe; micromachined probe		15. NUMBER OF PAGES 5		16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-1  
298-102

20020426 103

## FINAL REPORT

GRANT #: N00014-98-1-G-0677

PRINCIPAL INVESTIGATOR: Daniel van der Weide

INSTITUTION: University of Delaware (c/o University of Wisconsin)

GRANT TITLE: Nanoscale Field Localization for Manipulation and Probing of Computationally Interesting Biomolecules

AWARD PERIOD: 01 May 1998 - 13 Aug 2000

OBJECTIVE: In order to employ biomolecules in advanced computational structures, we need a deeper understanding of their structure and dynamics, as well as an electrical interface for communicating with them. To support this pursuit are applying newly developed micromachined silicon probes to probe protein function with minimal invasiveness.

APPROACH: We are designing, fabricating, and applying multifunctional scanning probe microscope (SPM) tips to probe single protein channels. We are using both photonic and electronic probes so we can examine local fluorescence as well as the electronic (i.e. conducting/non-conducting) state of ion channel proteins.

ACCOMPLISHMENTS: Several new and important results were enabled by this grant, which was to build a bioelectronic interface to single protein molecules. This interface consists of both environmental and mechanical supports for a membrane protein. While supported membranes are being studied and developed in many laboratories, single channel probing has been performed almost exclusively with fragile glass pipettes, or with cumbersome planar bilayer equipment, both of which are unsuitable for field deployment. Single channel recording cannot yet be accomplished with membranes on planar supports, although there are several startup companies attempting to achieve this goal. In this work, multifunctional scanned probe microscope tips were designed and fabricated to use in accessing the state of a membrane porin protein using microwave reflection and transmission, as well as fluorescent probing using photodiode probes.

Specific accomplishments were: development of a micromachined probe process and several probes to perform combined scanning force microscopy and microwave probing, development of a companion process for combined optical and scanning force probing, imaging of calcium waves in brain slices, and preliminary results on single-protein probing with both direct-current and high-frequency probes.

With new photodiode SPM tips, we can observe local optical phenomena such as fluorescence while simultaneously mapping the topography of a protein in a membrane. Although various groups have achieved each of these goals separately, no group has demonstrated the combined measurements needed to understand single channel responses more completely. Using new near-field microwave SPM tips, we are preparing to probe changes in both the conductance and capacitance of the channel, a significant advance in biochemical metrology. When the binding site is occupied, the channel changes its conformation and conductivity to a measurable extent, regardless of the charge of the analyte.

During the grant period, we built an inverted laser scanning confocal microscope with atomic force microscope (AFM) compatibility, then acquired a Park Scientific Bioprobe AFM to work with the confocal. We designed and constructed new planar horizontal bilayer fixtures to accommodate single proteins in black lipid membrane (BLM) for simultaneous optical and mechanical/electrical probing. Finally, and most importantly, we achieved preliminary results in probing single alpha-hemolysin porin proteins using both DC and radio-frequency techniques, in both reflection and in transmission. This was the primary goal of the proposal.

During this period we also collaborated with Prof. P.G. Haydon (University of Pennsylvania) to make single-channel calcium channel recordings in rat hippocampal neurons. Together we have also built a fluorescence microscope with a remote-controlled patch clamp manipulator for correlating conventional single-channel recordings with fluorescent probes and the microwave probes. This instrument can be controlled via an Internet interface, to enable biologists in other laboratory to eventually access our unique instrumentation.

**CONCLUSIONS:** A new type of electrical interface to single membrane proteins (porins) was developed with the support of this grant. Both microwave and optical scanning probe microscope tips were developed through a complex but high-yielding micromachining process, and near-field probing of single proteins was accomplished with probe tips of similar design. More opportunity to probe cells using force microscopy now exists, and should be the next step taken in this effort.

**SIGNIFICANCE:** Previous work on probing the states of individual membrane proteins used fragile probes and noise-sensitive techniques; ours paves the way for field-deployable sensing of protein activity at the single-channel level.

**PATENT INFORMATION:** A patent application on "Method and Apparatus for High Frequency Interfacing to Biochemical Membranes" was filed.

AWARD INFORMATION: ONR Young Investigator (1998), Alexander von Humboldt research fellowship (2000, postponed), promoted to Associate Professor (1998)

PUBLICATIONS AND ABSTRACTS (for total period of grant):

[1-11]

- [1] D. W. van der Weide, V. Agrawal, T. Bork, and P. Neuzil, "Localized circuit probing with a combined Schottky diode/scanning force microscope," in *1998 IEEE MTT-S International Microwave Symposium Digest*, vol. 3, R. Meixner, Ed. New York, NY, USA: IEEE, 1998, pp. 1341-4.
- [2] D. W. van der Weide, "Microscopes for the sub-visible: scanning the near field in the microwave through infrared," *Optics and Photonics News*, vol. 9, pp. 40-45, 1998.
- [3] T. Bork, V. Agrawal, B. Rosner, P. Gustafson, and D. W. van der Weide, "Shielded-tip/cantilever process and interface for multifunctional scanning," presented at Solid-State Sensor and Actuator Workshop, Hilton Head Island, SC, 2000.
- [4] A. Murakowski, M. Cywiak, B. Rosner, and D. W. van der Weide, "Far field optical imaging with subwavelength resolution," *Optics Communications*, vol. 185, pp. 295-303, 2000.
- [5] B. Rosner, T. Bork, V. Agrawal, P. Neuzil, and D. W. van der Weide, "Near-field optical mapping using cantilevered nanoscopic Schottky diode tips," presented at SPIE's 46th Annual Meeting International Symposium on Optical Science and Technology, San Diego, CA USA, 2001.
- [6] B. Rosner, T. Bork, V. Agrawal, and D. W. van der Weide, "Microfabricated silicon coaxial field sensors for near-field scanning optical and microwave microscopy," *To appear in Sensors and Actuators A*, 2002.
- [7] B. Rosner and D. W. van der Weide, "Feedback frequency mixing for non-contact SFM," *Submitted to Review of Scientific Instruments*, 2002.
- [8] D. W. van der Weide, K. Taylor, J. Peck, C. Wichaidit, S. Hagness, W. Cai, and R. Hamers, "Biomolecular contrast mechanisms and sensing techniques in the terahertz regime," presented at 9th International Conference on Terahertz Electronics, Charlottesville, 2001.
- [9] C. Wichaidit, J. R. Peck, L. Zhang, R. J. Hamers, S. C. Hagness, and D. W. van der Weide, "Resonant slot antennas as transducers of DNA hybridization: a computational feasibility study," presented at IEEE MTT-S International Microwave Symposium, Phoenix, AZ, 2001.
- [10] B. Rosner, J. Peck, and D. W. van der Weide, "Near field antennas integrated with scanning probes for THz to visible microscopy: Scale modeling and limitations on performance," *To appear in IEEE Transactions on Antennas and Propagation*, 2002.

- [11] B. T. Rosner, P. Kabos, D. F. Williams, and D. W. van der Weide, "Microfabricated silicon microwave near-field probes for circuit-probing," presented at Invited submission to American Society of Nondestructive Testing Spring Conference, Portland, OR, 2002.